and throat rugose; abdomen and ventral surface of thighs coarsely granular; outer edge of forearm and fourth finger with a conspicuous smooth-edged ridge of skin; a similar ridge along outer edge of tarsus and fifth toe; a long, pointed dermal flap on the heel; a transverse ridge above vent, several conspicuous tubercles below vent.

Color (in alcohol) light grayish brown above with irregular darker markings on the back; canthal ridge light; sides dark gray with small, irregular, white spots; ventrally whitish; throat with a few very small dark spots; ventral surface of hind limbs peppered with dark dots; dorsal surface of limbs cross-barred; posterior surface of thighs dark gray; transverse ridge above anus white edged with black; tubercles below anus white.

Snout to vent 61 mm.

*Paratypes.*—U.S.N.M. nos. 130216–17, from the type locality.

These agree with the holotype in all details save coloration. Both paratypes have about twelve narrow dark bars across the head and back. The white spots of the sides are more abundant than in the holotype, and in one (130216 similar markings are scattered over the posterior surface of the thigh.

U.S.N.M. 130217 is a male with a grayish nuptial pad on the dorsomedian surface of the first finger. Slitlike vocal sac openings are present along the sides of the floor of the mouth. Snout to vent is 54 mm.

U.S.N.M. 130216 is an adult female; snout to vent is 64 mm.

*Remarks.*—This species most closely resembles *R. javanus* Boettger. The dermal appendages at the heel and over the vent are identical in the two forms. Though a ridge of skin occurs along the outer edges of the forearm and tarsus of *javanus*, these ridges are apparently less well developed than in *baluensis*. The webbing of the hand is more extensive in *baluensis*, that of *javanus* failing to reach the disks of the outer fingers and present only at the base between the inner fingers. The snout of *javanus* is blunt or rounded and only feebly projecting instead of pointed and strongly projecting as in *baluensis*.

Of the Bornean forms of *Rhacophorus*, *baluensis* resembles *fasciatus* Boulenger (type locality Akar River, Sarawak) and *shelfordi* Boulenger (type locality, Mount Penrissen, Sarawak). Neither of these, however, has a pointed dermal appendage at the heel or a distinct ridge of skin on the tarsus. Furthermore the nostril is equidistant from the tip of the snout and the orbit in the species described by Boulenger instead of much nearer the tip of the snout as in *baluensis*.

**Rhacophorus spiculatus** (Smith)

Though referred to the genus *Philautus* by Smith (op. cit.), the extensive webbing and the presence of well-developed vomerine teeth suggest closer relationship to *Rhacophorus*. The conical tubercles of the limbs and infra-anal region recall *Rhacophorus everetti* of Palawan although the latter species has no tubercles on the dorsal surfaces of head and body.

## MALACOLOGY.—*The weight relations between shell and soft tissues during the growth of some fresh-water snails.* M. O. NOLAN and THEODOR VON BRAND,[1] National Microbiological Institute,[2] Bethesda, Md.

According to Thompson (1917), Nomura (1927), and Huxley (1932), the shell growth of snails is amenable to mathematical analysis. The shell of such snails as the Planorbidae can be considered as a logarithmic spiral, and its growth can be expressed adequately by the customary equation for allometric growth. So far no attempt seems to have been made to study whether fixed relationships exist between the amounts of shell material and soft tissues during the growth of snails. Such information, however, is of importance because physiological studies often involve a comparison in rates of certain processes, such as respiration, between various species or individuals of one species. Since the shell, though probably not entirely inert metabolically (von Brand, 1931; von Brand, Nolan, and Mann, 1948), certainly contributes at most a small fraction of the over-all metabolism, the quantitative weight relationships between shell and soft tissues assume a certain importance.

[1] The authors are indebted to Elizabeth M. Landry and Lawrence C. Pulley for technical assistance.

[2] Laboratory of Tropical Diseases.

## MATERIAL AND METHODS

The snails used in the present studies were as follows:

1. *Australorbis glabratus*. Five strains.

*a*. Venezuelan strain maintained in the laboratory since 1947. Rearing methods have been discussed previously (Nolan, Bond, and Mann, 1953). Number of snails studied, 246; diameter range, 5.7–27.3 mm; weight range, 28.7–2025.3 mgm.

*b*. Venezuelan red (pigment-free) mutant strain reared from a self-fertilized specimen which appeared in the stock colony mentioned above; maintained as a true-bred variety for more than 2 years. Number of snails studied, 265; diameter range, 4.7–23.8 mm; weight range, 38.6–1330.8 mgm.

*c*. "Wild" Venezuelan strain collected at Acequia El Cortijo, about 5 kilometers northeast of the town of Villa de Cura in the State of Aragua. We are greatly indebted to Dr. J. A. Jove and his coworkers of the Ministry of Health of Venezuela for supplying the snails. The day after collection they were wrapped in moist cotton, packed in empty paraffin milk cartons, and, traveling by airplane, were received in Washington, D. C., two days later. They were maintained in aquaria with food provided until studies were completed within approximately 1 to 2 weeks following the date of collection. Number of snails studied, 114; diameter range, 7.6–18.1 mm; weight range, 56.0–572.8 mgm.

*d* and *e*. We are indebted to Dr. Charles Dobrovolny for two "wild" Brazilian strains transported in moist condition by plane to this country. In the laboratory they were maintained in aquaria as above. Studies of one strain, collected near Church, Olinda, were completed within 2 to 3 weeks after arrival. Number of snails studied, 98; diameter range, 7.0–16.6 mm; weight range, 55.0–574.4 mgm. Studies of the other strain, collected at Barro Novo, Olinda, were completed within 3 to 4 weeks following their arrival. Number of snails studied, 54; diameter range, 13.9–24.0 mm; weight range 261.0–1341.7 mgm.

2. *Aplexa nitens* collected near Brownsville, Tex., and maintained in the laboratory since 1949. Number of snails studied, 173; diameter range, 8.0–23.1 mm; weight range, 22.7–682.0 mgm.

3. *Lymnaea stagnalis* colony derived from laboratory-reared specimens obtained from Dr. L. E. Noland, University of Wisconsin. Number of snails studied, 213; diameter range, 8.5–40.6 mm; weight range, 18.8–2980.5 mgm.

Prior to obtaining weight determinations, each snail was removed from water and the outside of the shell dried with filter paper. The snail was placed on dry filter paper and allowed to crawl around until it began to slowly retract within its shell. Small pieces of filter paper were then inserted into the shell aperture and all visible moisture was removed. The total retraction of the body at this stage was just under half the length of the outer whorl. The water remaining between tissues and shell behind the foot and mouth parts was considered as part of the normal fresh weight of the snail. Weight was determined on an analytical balance. The snail was returned to water in order to ready it for removal of the body from the shell.

Before removing the body the snail was held with forceps and dipped into boiling water. With *Australorbis* it was important that the snail not be retracted before immersion in boiling water and the period of immersion be brief, 5 seconds or less. With *Aplexa* the period of immersion was 20 seconds and with *Lymnaea* 15 seconds. The snail was then held in the fingers under a dissecting microscope. A thin pliable hooked wire (fitted with a handle) was inserted into the muscular pharynx and the body pulled out in toto with gentle firmness, the pressure of the pull being in the direction of the curvature of the shell. The water that remained in the shell was driven off by heating in an oven at 100° to 105° C. The drying period was 1 to 2 hours for all specimens except large *Lymnaea* which required 3 hours for complete drying. The shell was cooled in a desiccator before being weighed on an analytical balance. Separate determinations on pieces of shell broken from the living animals showed that the shell contained very little water. The dried shell weight is therefore for all

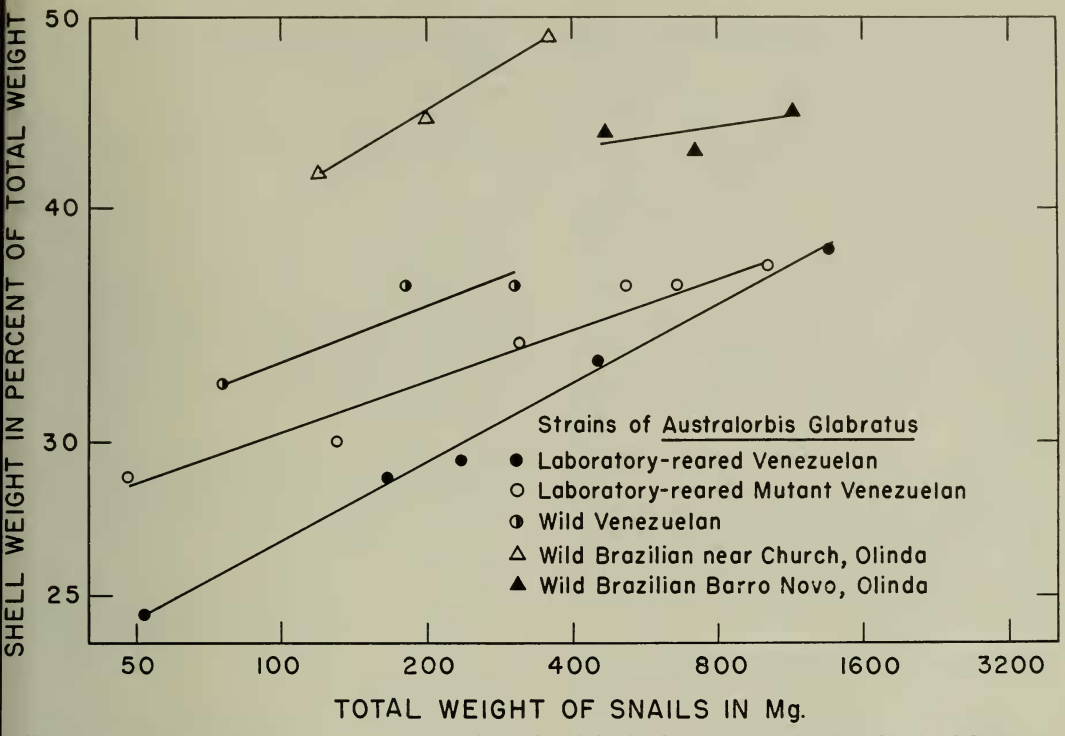


FIG. 1.—Relations between shell weight and total weight in five strains of *Australorbis glabratus*.

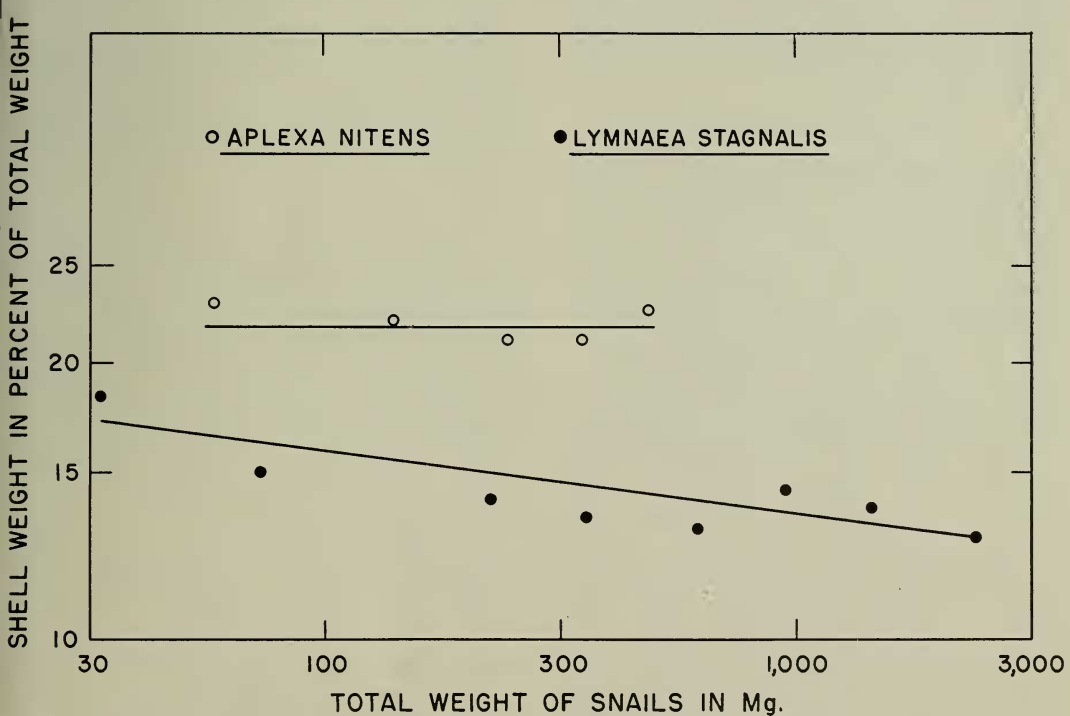


FIG. 2.—Relations between shell weight and total weight in *Aplexa nitens* and *Lymnaea stagnalis*.

practical purposes identical with the weight of the fresh shell from which the excess water has been wiped off.

Sliding vernier calipers were used to determine diameter of the shell.

In order to determine the area of the aperture, a section of the last whorl was fixed with duco cement on a slide insuring that the aperture was lying flat on the slide. By means of a microprojector the aperture was projected at a magnification of 7.5 × on graph paper ruled in square millimeters, and its circumference outlined. The area was then determined by counting the squares.

Thickness of the shell was determined by isolating a fraction of the shell a few millimeters from the aperture, fixing it as above on a slide and measuring the thickness of the calcareous layer by means of an ocular micrometer at a magnification of 75 ×.

For the purpose of discussion the snails of each series were divided into arbitrary size groups in order to provide sufficient material for a valid average. The number of snails within these groups varied from series to series depending upon the total number of snails available but were similar in all groups of one series.

## RESULTS AND DISCUSSION

Fig. 1, summarizing the results obtained with five strains of *Australorbis glabratus*, shows that in all cases the relative shell weight increased with increasing size of the snails and that, upon plotting on a double logarithmic scale, straight lines resulted. This then proves that the relations between shell and soft tissues during growth are those of allometric growth. The slope of the curves differs in the various strains, and it is quite evident that the wild strain had heavier shells than the laboratory-reared ones. It is also evident that the shells of the Brazilian snails were heavier than those of the Venezuelan strain. It should be realized that the above relationship holds only when averages of fairly large numbers are compared. The individual variations were quite marked. This is illustrated by Table 1, which presents a more detailed summary of our data for one strain. It is evident

Table 1.—Relations between Total Weight and Relative Shell Weight in the Laboratory-reared Strain of Australorbis glabratus.

(In this and in Table 2 the figure following the ± sign is the standard error of the mean.)

| Number of snails | Total weight of snail in milligrams | | Shell weight in percent of total weight | |
|---|---|---|---|---|
| | Average | Range | Average | Range |
| 57 | 52.3 ± 2.2 | 28.7– 99.9 | 24.8 ± 0.49 | 18.3–36.7 |
| 30 | 166.3 ± 4.2 | 100.4– 199.1 | 29.0 ± 0.69 | 21.2–38.6 |
| 46 | 237.7 ± 4.1 | 200.8– 298.1 | 29.6 ± 0.44 | 23.8–36.7 |
| 42 | 442.4 ± 14.6 | 303.8– 598.9 | 33.1 ± 0.51 | 26.4–39.8 |
| 40 | 800.9 ± 20.9 | 600.2– 996.3 | 35.6 ± 0.76 | 26.3–49.3 |
| 31 | 1314.8 ± 52.2 | 1013.7–2025.3 | 37.9 ± 0.73 | 30.5–46.9 |

Table 2.—Relations between Diameter, Area of Aperture, and Thickness of Calcareous Shell Layer in Several Species of Aquatic Pulmonate Snails

| Species | Average diameter (in mm.) | Average area of aperture (in mm²) | Average thickness of calcareous layer of last whorl (in mm.) |
|---|---|---|---|
| *Australorbis glabratus* small | 6.9 ± 0.20 | 1.9 ± 0.15 | 0.03 ± 0.002 |
| *Australorbis glabratus* large | 24.8 ± 0.55 | 16.1 ± 0.72 | 0.18 ± 0.017 |
| Ratio small:large *Australorbis* | 1:3.6 | 1:8.5 | 1:6.0 |
| *Aplexa nitens* small | 9.2 ± 0.26 | 2.1 ± 0.17 | 0.04 ± 0.005 |
| *Aplexa nitens* large | 21.6 ± 0.35 | 13.0 ± 0.58 | 0.11 ± 0.001 |
| Ratio small:large *Aplexa* | 1:2.3 | 1:6.2 | 1:2.7 |
| *Lymnaea stagnalis* small | 9.8 ± 1.00 | 2.1 ± 0.38 | 0.03 ± 0.004 |
| *Lymnaea stagnalis* large | 39.0 ± 0.36 | 47.1 ± 2.22 | 0.10 ± 0.001 |
| Ratio small:large *Lymnaea* | 1:4.0 | 1:22.4 | 1:3.3 |

that while the difference in shell weight is not significant in all instances when two successive size groups are considered, the differences between some successive groups are significant, and when the smallest snails are compared with the largest ones, the difference is highly significant.

One strain each of *Aplexa nitens* and *Lymnæa stagnalis* was studied (Fig. 2). In the former species the values obtained could best be expressed by a horizontal straight line, that is, the percentage shell weight was about constant throughout the size range studied. In *Lymnaea*, on the other hand, the points were scattered around a declining straight line, or in other words, the relative shell weight had a tendency to decrease with increasing size.

The difference between successive groups was in general not significant statistically. However, the difference between the average shell weight of the smallest snails (18.1 ± 0.38 percent) and of the largest specimens (12.9 ± 0.09 percent) was definitely significant indicating that the above relationship is real.

Several reasons may be responsible for the different pattern in the weight relationships between shell and soft tissues (evidently the relative weight of the soft tissues is the reciprocal of the values discussed above for the relative shell weight). Two of the possible reasons have been singled out for study. In all species of snails under consideration the whorls became increasingly larger, the larger the snail was and this increase in size occurred in all spatial dimensions. As a consequence the proportion between the bulk of tissue and its surface (which corresponds essentially to the inner surface of the shell) is shifted with increasing growth, the bulk of tissue increasing faster than the surface. This is shown in Table 2 where measurements are given for the diameter of the snail and the area of the aperture. It is evident that in all species the aperture became relatively larger, and that this feature was least developed in *Aplexa*. This point then would evidently tend to lower the percentage weight of the shell. It is counterbalanced by an increased thickness of the calcareous layer of the shell in the wider whorls. Table 2 shows that this increase in thickness was most pronounced in *Australorbis* and least pronounced in *Aplexa*. The increase in thickness of the shell tends, of course, to increase the relative shell weight. The relative increase in diameter was similar in the groups of *Australorbis* and *Lymnaea* under consideration; the differences in the ratios of diameter, aperture, and shell thickness are clear cut. It is believed that the balance between these points is essentially responsible for the differences in shell tissue proportions demonstrated between individuals belonging to one species but differing in size and for the different patterns observed in various species.

## SUMMARY

The relationships between shell and soft tissues during the growth of three species of aquatic pulmonate snails are those of allometric growth. The percentage shell weight increases progressively with increasing size in *Australorbis glabratus*, remains constant in *Aplexa nitens*, and declines in *Lymnaea stagnalis*. These differences are related to differences in the change in ratio between the bulk of the tissues and their surface during growth and to differences in the increase in thickness of the calcareous layer of the shell in older and younger whorls.

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